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## Comparison of microbial communities associated with three Atlantic ultramafic hydrothermal systems

Erwan G. Roussel<sup>1,\*</sup>, Cécile Konn<sup>2</sup>, Jean-Luc Charlou<sup>2</sup>, Jean-Pierre Donval<sup>2</sup>, Yves Fouquet<sup>2</sup>, Joël Querellou<sup>1</sup>, Daniel Prieur<sup>1</sup>, Marie-Anne Cambon Bonavita<sup>1</sup>

<sup>1</sup> Laboratoire de Microbiologie des Environnements Extrêmes, UMR 6197, Université de Bretagne Occidentale, Ifremer, CNRS, Institut Universitaire Européen de la Mer, Plouzané, France

<sup>2</sup> Département Géosciences Marines, Ifremer, Centre de Brest, Plouzané, France

\*: Corresponding author : Erwan G. Roussel, Tel.: +44 29 2087 4488 ; fax: +44 29 2087 4326 ;

e-mail address : [rousseleg@cardiff.ac.uk](mailto:rousseleg@cardiff.ac.uk)

### Abstract :

The distribution of *Archaea* and methanogenic, methanotrophic and sulfate-reducing communities in three Atlantic ultramafic-hosted hydrothermal systems (Rainbow, Ashadze, Lost City) was compared using 16S rRNA gene and functional gene (*mcrA*, *pmoA* and *dsrA*) clone libraries. The overall archaeal community was diverse and heterogeneously distributed between the hydrothermal sites and the types of samples analyzed (seawater, hydrothermal fluid, chimney and sediment). The Lost City hydrothermal field, characterized by high alkaline warm fluids (pH>11;  $T<95^{\circ}\text{C}$ ), harbored a singular archaeal diversity mostly composed of unaffiliated *Methanosarcinales*. The archaeal communities associated with the recently discovered Ashadze 1 site, one of the deepest active hydrothermal fields known (4100 m depth), showed significant differences between the two different vents analyzed and were characterized by putative extreme halophiles. Sequences related to the rarely detected *Nanoarchaeota* phylum and *Methanopyrales* order were also retrieved from the Rainbow and Ashadze hydrothermal fluids. However, the methanogenic *Methanococcales* was the most widely distributed hyper/thermophilic archaeal group among the hot and acidic ultramafic-hosted hydrothermal system environments. Most of the lineages detected are linked to methane and hydrogen cycling, suggesting that in ultramafic-hosted hydrothermal systems, large methanogenic and methanotrophic communities could be fuelled by hydrothermal fluids highly enriched in methane and hydrogen.

**Keywords :** *Archaea* ; hydrothermal vent ; Mid-Atlantic Ridge ; 16S rRNA gene ; Sediment ; ultramafic

### Introduction

Deep-sea hydrothermal environments are characterized by intense physico-chemical gradients providing a large range of habitats for chemolithoautotrophic microorganisms (Kelley *et al.*, 2002). Most of the studies of microbial diversity associated with deep-sea hydrothermal environments have mainly investigated basaltic-hosted hydrothermal systems (Kelley *et al.*, 2002). However, a few studies showed that ultramafic-hosted hydrothermal systems contained specific microbial communities (Brazelton *et al.*, 2006; Perner *et al.*, 2007; Voordeckers *et al.*, 2008; Flores *et al.*, 2011). To date, only three ultramafic sites were fully described on the Mid-Atlantic Ridge (Rainbow, Lost City and Logatchev), and were characterized by high concentrations of methane and hydrogen, in contrast with basaltichosted hydrothermal systems (Kelley *et al.*, 2001; Charlou *et al.*, 2002; Schmidt *et al.*, 2007). Moreover, Ashadze, a novel hydrothermal site, was recently reported on the Mid-Atlantic Ridge (MAR) (Bel'tenev *et al.*, 2005; Charlou *et al.*, 2007; Fouquet *et al.*, 2007; Mozgova *et al.*, 2008; Bassez *et al.*, 2009; Charlou *et al.*, 2010). This part of the MAR is characterized by rock compositions indicating that anomalously enriched mantle domains are involved in the melting region (Dosso *et al.*, 1993), and also by numerous outcrops of serpentinized mantle-derived rocks (Bougault *et al.*, 1993; Cannat *et al.*, 1997). However, these ultramafic systems expelling fluids characterized by moderate to high temperatures are also probably linked to magmatic heating processes (Allen & Seyfried, 2004). Ultramafic hydrothermal fluids are

highly enriched in abiogenic methane and hydrogen as a result of serpentinization reactions between the ultramafic rocks and seawater (Holm & Charlou, 2001; Charlou *et al.*, 2002; Allen & Seyfried, 2004), and could therefore supply twice as much chemical energy as basaltic-hosted hydrothermal systems (McCollom, 2007). Hence, most of the prokaryotes found at these sites seemed to be related to methane and hydrogen cycling (Boetius, 2005; Perner *et al.*, 2007; Voordeckers *et al.*, 2008; Flores *et al.*, 2011).

A large number of microbial communities from hydrothermal environments could be fuelled by inorganic compounds (Amend & Shock, 2001). Although these microbial communities occupy both aerobic and anaerobic habitats, anaerobic hyper/thermophilic *Archaea* are reported to be usually associated with the hottest parts of these environments (Kelley *et al.*, 2002; Schrenk *et al.*, 2003; Takai *et al.*, 2004a), some of which could be entrained by hydrothermal fluids from subsurface ecosystems (Deming & Baross, 1993; Holden *et al.*, 1998; Summit & Baross, 1998). Moreover, it was suggested that *Archaea* could encompass up to 33-50% of the total microbial community in deep-sea hydrothermal environments (Harmsen *et al.*, 1997; Nercessian *et al.*, 2003).

Although an increasing number of thermophilic prokaryotes are cultivated from hydrothermal environments (Huber *et al.*, 2002; Miroshnichenko & Bonch-Osmolovskaya, 2006; Reysenbach *et al.*, 2006; Wagner & Wiegel, 2008; Slobodkina *et al.*, 2009), molecular phylogenetic approaches have revealed several new uncultivated lineages (Takai & Horikoshi, 1999; Nercessian, 2003; Kormas *et al.*, 2006; Moussard *et al.*, 2006a).

Metagenomic approaches and functional gene analyses have also contributed to the characterization of metabolic and physiological properties of these communities (Nercessian *et al.*, 2005; Moussard *et al.*, 2006b; Moussard *et al.*, 2006c). However, to our knowledge, rRNA-based molecular approaches have seldom been used to compare the microbial diversity from multiple different hydrothermal sites (López-García *et al.*, 2003a; López-García *et al.*, 2003b; Voordeckers *et al.*, 2008; Flores *et al.*, 2011).

In the present study, we characterized the molecular genetic diversity, using 16S rRNA gene and functional genes of methanogens, methanotrophs and sulfate-reducers, associated with

three ultramafic-hosted hydrothermal sites: Rainbow, Lost City and Ashadze. As these hydrothermal fluids are highly enriched in methane and hydrogen, these environments could harbour specific prokaryotic communities possibly associated with potential subsurface chemolithoautotrophic ecosystems. Hence, the aim of this study was to compare the microbial communities of these ultramafic-hosted hydrothermal sites using molecular genetic methods, in order to correlate their phylogeny with ecological niches.

## Materials and methods

### Site location and sampling techniques

Fluid, chimney and sediment samples were collected during the scientific cruises EXOMAR (2005), SERPENTINE (2007) and MoMARDREAM-Naut (2007) conducted with the R. V. “*L’Atalante*” and “*Pourquoi pas ?*” and using the ROV “*Victor 6000*” and DSV “*Nautilie*”. The three hydrothermal fields explored, Rainbow (36°13’N; 33°54’W; ~ 2300 m depth), Lost City (30°07’N; 42°07’W; ~ 750 m depth) and Ashadze 1 (12°58’N; 44°51’W; ~ 4090 m depth) were all located along the Mid-Atlantic Ridge (MAR), although Lost City and Ashadze were further from the axis (Fig. 1a).

Fluid samples from Rainbow, Lost City and Ashadze were collected respectively from the “*thermitière*” chimney (36°13’76”N; 33°54’16”W; 2294 m depth, Fig. 1e), from a flange near the EXOMAR 11 Marker (30°07’43” N; 42°07’16”W; 748 m depth, Fig. 1c), and from two chimneys in the SE2 area at Ashadze 1 site (12°58’33”N; 44°51’78”W; 4097 m depth, Fig. 1f). Chimney samples were also retrieved from the two chimneys in the SE2 area of Ashadze 1 site where the fluids were previously collected. The sediment samples were retrieved from the Rainbow site close to the active hydrothermal area (36°13’76”N; 33°54’04”W; 2287 m depth, Fig. 1d) and from the immediate periphery of the Lost City site (30°07’57” N; 42°07’05”W; 752 m depth, Fig. 1b).

In order to describe the microbial communities from the surrounding seawater, the water column from the Rainbow site (36°13’76”N; 33°54’06”W; 2291 m depth) was also sampled.

All fluid samples were collected using titanium syringes and analyzed as described elsewhere (Charlou *et al.*, 2002). On board, the fluid samples were immediately removed aseptically from the titanium syringes and stored at  $-80^{\circ}\text{C}$  for molecular genetic analyses. On board the sediment cores ( $\sim 20$  cm in length, 5 cm diameter) collected from the Rainbow site, using a push-core device operated by the arm of the DSV "*Nautilé*", were sectioned in three equal samples and were designated as top, middle and bottom. The sediment surface sample from the Lost City site was collected using PSDE system (Fig. 1b, Kato C., unpublished). The chimney fragments were collected in biobox and sediment samples were stored aseptically at  $-80^{\circ}\text{C}$  for molecular genetic analyses.

#### **DNA extractions and PCR amplification**

To avoid contaminations, all manipulations were carried out in a PCR cabinet (Biocap<sup>TM</sup> RNA/DNA, erlab<sup>®</sup>), using Biopur<sup>®</sup> 1.5 mL Safe-Lock micro test tubes (Eppendorf<sup>TM</sup>), Rnase/Dnase Free Water (MP Biomedicals<sup>TM</sup>) and UV-treated ( $>60$  min) plasticware and pipettes.

DNA extractions from fluids were performed from 50 mL of fluid left to thaw on ice prior centrifugation (15000 g for 60 min). Supernatant was carefully discarded and DNA was extracted from the pellet, following a modified FastDNA<sup>®</sup> Spin Kit for Soil (Bio101 Systems, MP Biomedicals<sup>TM</sup>) protocol (Webster *et al.*, 2003; Roussel *et al.*, 2009a). The DNA extractions from sediments and chimney fragments were also performed using the modified FastDNA<sup>®</sup> Spin Kit for Soil as described elsewhere (Roussel *et al.*, 2009a).

All amplifications were performed using a "GeneAmp PCR system" 9700<sup>®</sup> (Applied Biosystems<sup>TM</sup>). All PCR mixtures (50  $\mu\text{L}$ ) contained 5  $\mu\text{L}$  of DNA template, 1X Taq DNA polymerase buffer (MP Biomedicals<sup>TM</sup>), 1  $\mu\text{L}$  of dNTP (10 mM of each dATP, dCTP, dGTP and dTTP), 10  $\mu\text{M}$  of each primer and 0.5  $\mu\text{L}$  of Taq DNA polymerase (MP Biomedicals<sup>TM</sup>). Negative controls were also carried out with DNA extractions performed without any sample. For all controls, no PCR products were detected. Inhibition of PCR amplification by soluble

contaminants in the DNA extracts was also tested as described elsewhere (Juniper *et al.*, 2001).

Archaeal 16S rRNA gene amplification was conducted by nested PCR with combination of primers A8f (5'-CGG TTG ATC CTG CCG GA-3') and A1492r (5'-GGC TAC CTT GTT ACG ACT T-3') in the first round (Teske *et al.*, 2002; Lepage *et al.*, 2004), and with A344f (5'-AYG GGG YGC ASC AGG SG-3') and A915r (5'-GTG CTC CCC CGC CAA TTC CT-3') in the second round (Stahl & Amann, 1991; Sørensen *et al.*, 2004). PCR cycles for the first round (A8f/A1492r), and for the second round (A344f/A915r) were as previously described (Roussel *et al.*, 2009a). To minimize PCR bias, five independent PCR products from the first round were pooled and purified (QIAquick PCR purification Kit; Qiagen™) and used as template for the second round. This nested PCR was necessary to obtain visible PCR products on a 0.8% (w/v) agarose gel stained with ethidium bromide.

A portion of the *mcrA* gene was amplified using the ME primers (Hales *et al.*, 1996) with the following reaction conditions as described elsewhere (Roussel *et al.*, 2009a). A fragment of the *pmoA* gene was amplified using the pmoA189-mb661 primer couple (Holmes *et al.*, 1995; Costello & Lidstrom, 1999) with the following reaction conditions: 1 cycle of 4 min at 92°C, 35 cycles of 1 min at 92°C, 1.5 min at 55°C and 1 min at 72°C, and 1 cycle of 9 min at 72°C. A portion of the *dsrA* gene was amplified using the DSR1F+ and DSR-R primers (Kondo *et al.*, 2004) with the following reaction conditions: 1 cycle of 5 min at 94°C, 35 cycles of 30 s at 94°C, 30 s at 54°C and 2.5 min at 72°C, and 1 cycle of 8 min at 72°C. For all functional genes, two rounds with the previous reaction conditions were required to obtain visible amplification products. An aliquot (5 µL) of three pooled PCR products of the primary amplification was used as template for the second amplification round.

## CM-DGGE analysis

In order to obtain the general archaeal 16S rRNA gene diversity associated with the hydrothermal environment and to compare it with the seawater diversity, a preliminary CM-DGGE analysis was performed as described elsewhere (Roussel *et al.*, 2009b).

After amplification of the nested PCR products, using two different fluorescent reverse labelled (Cy3 or Cy5) primers from total DNA from either a hydrothermal sample or seawater, these were pooled and loaded into the same lane. Archaeal 16S rRNA gene amplification was performed with primers Saf-PARCH 519r, labelled with either Cy3 (hydrothermal samples) or Cy5 (seawater), following touchdown PCR protocol as previously described (Nicol *et al.*, 2003). All manipulations were performed in the dark. The PCR products were analyzed by DGGE using a DCode Universal Mutation Detection System<sup>®</sup> (BioRad<sup>™</sup>) on a 1 mm thick (16 × 16 cm) 8% (w/v) polyacrylamide gel (acrylamide/bisacrylamide, 40%, 37.5:1, BioRad<sup>™</sup>) with a denaturant gradient between 30 and 70% prepared with 1 × TAE buffer (pH 8, 40 mM Tris Base, 20 mM acetic acid, 1 mM EDTA, MP Biomedicals<sup>™</sup>) and poured with a "Gradient maker" (Hoefer SG30<sup>®</sup>). Electrophoresis was carried in 1 × TAE buffer at 60°C for 330 min at 200 V (initially at 80 V for 10 min). The gel was scanned using a Phosphorimager Typhoon 9400<sup>®</sup> (Amersham Biosciences<sup>™</sup>).

### Cloning and sequencing

Fourteen 16S rRNA gene, one *dsrA* gene, four *mcrA* gene, and eight *pmoA* gene clone libraries were constructed. To minimize PCR bias (Polz & Cavanaugh, 1998), five independent PCR products were pooled, purified (QIAquick PCR purification Kit; Qiagen<sup>™</sup>), and cloned into *Escherichia coli* (XL10-Gold; Stratagene<sup>™</sup>) using the pGEM-T Easy vector system I (Promega<sup>™</sup>) following the manufacturer's instructions. Positive transformants were screened by PCR amplification of the insert using the vector-specific M13 primers. Plasmid extraction, purification and sequencing of the insert were carried out by the sequencing Ouest-Genepole platform<sup>®</sup> of Roscoff Marine laboratory (France).

### Phylogenetic analysis and statistical analyses

Chimeras (Cole *et al.*, 2003) were excluded from the clone libraries and a total of 759 sequences (including those from the 16S rRNA gene and functional genes) were used for further phylogenetical analysis. The phylogenetic placement was carried out using NCBI

BLAST search program within GenBank (<http://www.ncbi.nlm.nih.gov/blast>) (Altschul *et al.*, 1990). The 16S rRNA gene sequences (~553 bases) were then edited in the BioEdit 7.0.5.3 program (Hall, 1999) and aligned using CLUSTALW (Thompson *et al.*, 1994). The phylogenetic trees were constructed by the PHYLO\_WIN program ([http:// pbil.univ-lyon1.fr/](http://pbil.univ-lyon1.fr/)) (Galtier *et al.*, 1996) with Neighbour-Joining method (Saitou & Nei, 1987) and Jukes and Cantor correction. The nonchimeric *mcrA* (~0.76 kb), *pmoA* (~0.51 kb) and *dsrA* (~0.22 kb) sequences were translated into amino acids using BioEdit and then aligned using CLUSTALW, and the PHYLO\_WIN program with Neighbour-Joining algorithm, and PAM distance (Dayhoff *et al.*, 1978) was then used for phylogenetic tree construction. For the entire phylogenetic reconstruction, the robustness of inferred topology was tested by bootstrap resampling (1000), values over 50% are shown on the trees. The richness from the clone libraries was estimated, with the rarefaction curves at 99%, 97% and 95% sequence identity levels, using the DOTUR program (Schloss & Handelsman, 2005). Operational taxonomic units (OTUs), using a 95% or 97% sequence similarity, were generated with the SON program (Schloss & Handelsman, 2006), and the percentage of coverage (Cx) of the clone libraries was calculated by Good's method (Good, 1953) as described by Singleton and colleagues (Singleton *et al.*, 2001). Statistical estimators, the significance of population differentiation among clone libraries ( $F_{ST}$ ) (Martin, 2002), and the exact tests of population genetic differentiation (Raymond & Rousset, 1995), were calculated using Arlequin 3.11 (Excoffier *et al.*, 2005).

### **Nucleotide sequence accession numbers**

The sequences are available from GenBank database under the following accession numbers and names: 16S rRNA gene (FN650174 to FN650288), *mcrA* gene (FN650315 to FN650322), *dsrA* (FN650289 to FN650291) and *pmoA* (FN650292 to FN650314).



## Results

### Site description

A total of 15 samples encompassing fluids, chimney fragments and sediments, were retrieved from three Atlantic ultramafic-hosted hydrothermal sites: Rainbow, Lost City and Ashadze (Fig. 1). The dilution of the hydrothermal fluid sample was estimated according to pH measurements. Overall, the three sites had much higher hydrogen (<16 mM) and abiogenic methane (<2.5 mM) concentrations than the MAR basaltic-hosted hydrothermal sites (Charlou *et al.*, 2010).

All the hydrothermal fluid samples from the Rainbow site were retrieved from the “thermitière” chimney group (Fig. 1e), except the “PP27 swarm” sample which was obtained in close proximity to a shrimp swarm on the side of the PP27 chimney. The “thermitière” chimney group was composed of both diffuse and black smoker venting. The Rainbow sediment samples were retrieved nearby the hydrothermal chimneys and were predominantly made of pelagic sediment (98% calcite) with a small amount of hematite, indicating a small hydrothermal contribution (Fig. 1d). For this study, the maximum temperature measured at Rainbow was 324°C, and the less diluted hydrothermal fluid analyzed had a pH of 3.40 (Fig. 2b), and high concentrations of hydrogen (>10 mM), carbon dioxide (17 mM), iron (>17 mM) and methane (>1mM) (Charlou *et al.*, 2010).

The Lost City fluid samples were obtained from one of the hottest venting areas of this site, which was located above a flange (Fig. 1c). To date, Lost City is a unique off-axis hydrothermal site expelling fluids with a high pH (~ 11), as opposed to the other known ultramafic environments that are acidic (Rainbow, Ashadze pH = ~ 3). The maximum temperature recorded at Lost City (93°C) was lower than for Rainbow and Ashadze. The less diluted hydrothermal fluid analyzed had a pH of 11.75 (Fig. 2a), and high concentrations of hydrogen (>7 mM) and methane (0.9 mM).

Ashadze, a hydrothermal field that was recently explored for the first time during the French-Russian Serpentine cruise (Fouquet *et al.*, 2008), is one of the deepest active black smoker

fields discovered so far (4100 m depth). Ashadze is characterized by an ultramafic rock environment (Charlou *et al.*, 2007; Fouquet *et al.*, 2007; Fouquet *et al.*, 2008). Several groups of active one to two meter high chimneys were observed at Ashadze 1 site. The fluid and chimney fragments were obtained from two different active chimneys in a unique group near the SE-2 marker (Fig. 1f). For this study, the maximum temperature measured at Ashadze was 353°C. The less diluted hydrothermal fluid analyzed had a pH of 4.02 (Fig. 2b), and high concentrations of hydrogen (>10 mM), carbon dioxide (>2.5 mM), iron (7.3 mM) and methane (>0.80 mM).

### **Archaeal 16S rRNA gene analyses**

*CM-DGGE.* All the 16S rRNA gene PCR products from all the samples were screened by Co-Migration DGGE (CM-DGGE) prior cloning, in order to estimate the archaeal phylogenetic diversity of each hydrothermal sample and to compare it directly with the seawater diversity (Fig. 2a). Band pattern intensities from all Lost City samples, and from the less diluted hydrothermal fluids, were weaker than for all the other samples, suggesting a lower biomass and/or high concentration of PCR inhibitors (Fig. 2a). The archaeal seawater CM-DGGE band pattern was different from all the hydrothermal fluid and chimney band patterns (Fig. 2a), suggesting low levels of seawater contamination. The band patterns from hydrothermal samples were mostly composed of DGGE fragments with higher melting points, a probable consequence of higher GC content of the 16S rRNA gene. The high-GC content of these 16S rRNA gene sequences indicates that the *Archaea* could be hyper/thermophiles (Kimura *et al.*, 2006), as also suggested by the several putative hyper/thermophilic lineages detected in the clone libraries from hydrothermal fluids and chimneys (*Archaeoglobales*, *Methanococcales*, *Thermococcales*, *Methanopyrales*, *Desulfurococcales*, *Nanoarchaeota*, DHVE; Fig. 2b).

*Clone libraries.* After technical optimization and removal of soluble PCR inhibitors and in order to amplify sufficient PCR product for cloning, archaeal amplifiable DNA from all samples was retrieved by nested PCR. However, no sufficient amplified PCR product was

obtained for cloning from the less diluted fluid samples (pH 11.75) and from the chimney samples from Lost City. Fourteen different 16S rRNA gene clone libraries were constructed, representing a total of 610 sequences. The coverage values for the 16S rRNA gene clone libraries ranged from 68 to 97%, based on a 97% sequence similarity level (Fig. 2b). On the whole, rarefaction curves were asymptotic for all clone libraries, based on a 95% sequence similarity level, confirming sufficient sampling effort (Fig. S1).

The overall archaeal diversity analyzed was similar to previous studies (e.g. Brazelton *et al.*, 2006; Flores *et al.*, 2011) and very heterogeneously distributed between the sites (Lost City, Rainbow, and Ashadze) and between types of samples (seawater, hydrothermal fluid, chimney and sediment). The number of OTUs per clone library ranged from five to nineteen, based on a 95% genus level of phylotype differentiation (Schloss & Handelsman, 2004), and the Shannon-Wiener index of diversity ranged between 0.63 and 3.04 (Fig. 2b). The archaeal diversity indices of all the samples were in the same range, except for the fluid associated with Lost City which, as previously described (Schrenk *et al.*, 2004), displayed the lowest detectable diversity (Fig. 2a and 2b). On average, the hydrothermal samples contained six different lineages, except for Lost City (Fig. 2a and 2b), which is also in agreement with most published studies on hydrothermal environments (e.g. Takai *et al.*, 2001; Nercessian *et al.*, 2003; Schrenk *et al.*, 2003; Schrenk *et al.*, 2004; Takai *et al.*, 2004b; Kormas *et al.*, 2006; Page *et al.*, 2008; Nunoura *et al.*, 2010). All the 16S rRNA gene sequences obtained from the clone libraries were assigned to 95 OTUs, based on a 95% sequence similarity level, forming a total of 21 different phylogenetic lineages (Fig. 2b, 3a and 3b). On the whole, 16S rRNA gene sequences were related to *Euryarchaeota* (51%), *Crenarchaeota* (48%) and *Nanoarchaeota* (1%). The 16S rRNA gene clone libraries obtained from hydrothermal samples (fluid and chimney) were dominated by sequences related to *Euryarchaeota* (69%), whereas sequences related to *Crenarchaeota* were a majority in the sediment (92%) and seawater samples (70%) (Fig. 2b). Seven of the fifteen *Euryarchaeota* lineages detected had at least one known cultured representative, and six of these seven had known thermophilic *Archaea* (*Halobacteriales*, DHVE2, *Archaeoglobales*, *Methanococcales*, *Thermococcales*,

*Methanopyrales*). Although three of the five *Crenarchaeota* lineages detected had at least one cultured representative, only one was known to be thermophilic (*Desulfurococcales*). Moreover, Marine Group I (MG-I) *Archaea* had the highest intra-lineage diversity representing 25 OTUs based on a 95% genus level of phylotype differentiation.

### Functional gene clone libraries

*Diversity of mcrA gene.* The operon coding for the MCR-I, which includes McrA subunit, is found in all known methanogens (Reeve *et al.*, 1997). Four *mcrA* clone libraries were obtained from sediment, fluid and chimney samples from Ashadze and Rainbow sites. Although detected by previous studies (Kelley *et al.*, 2005), no *mcrA* gene sequences were detected from Lost City samples. The diversity of the four *mcrA* libraries was limited to sequences related to the H<sub>2</sub>/CO<sub>2</sub> methanogens *Methanopyrales* and *Methanococcales* orders (Fig. 4a), congruently with the 16S rRNA gene clone libraries (Fig. 3a). *mcrA* gene sequences affiliated to *Methanopyrales* were only detected at Rainbow. Moreover, the *mcrA* gene sequences from Rainbow and Ashadze matched the two groups of uncultured methanogenic *Archaea* previously retrieved from Rainbow (Nercessian *et al.*, 2005).

*Diversity of dsrA gene.* Sequences coding for the *dsrA* gene were only retrieved from Ashadze chimney 1 (Fig. 4b). *dsrA* gene sequences were previously detected in chimney samples from Lost City (Gerasimchuk *et al.*, 2010), and in sediments from Rainbow (Nercessian *et al.*, 2005). All *dsrA* gene sequences detected from Ashadze site were all related to sequences from marine sediments and East-Pacific Rise hydrothermal vents, as a probable consequence of a lack of *dsrA* gene sequences from the MAR in the databases. *dsrA* gene sequences were mainly affiliated to sequences from the *Desulfobulbaceae* family (Fig. 4b).

*Diversity of pmoA gene.* The *pmoA* gene was the most widespread functional gene detected, as a PCR amplification was obtained on eight out of the fifteen samples tested (Fig. 2b and 4c). The phylogeny of the *pmoA* gene is usually poorly resolved, the bacterial *pmoA* gene being distantly related to the ammonia monooxygenase subunit A (*amoA*) (Holmes *et al.*,

1995; Nicol & Schleper, 2006), as revealed by incongruence between tree topologies performed with different phylogenetic methods. However, two groups of *pmoA* sequences from Rainbow fluids and Ashadze chimney samples clustered (cluster *pmoA* 1 and cluster P-A) with sequences related to thermophilic methylotrophs (Inagaki *et al.*, 2003; Hirayama *et al.*, 2007)(Fig. 4c). Moreover, *pmoA* gene sequences from sediments from Rainbow grouped into two major clusters (cluster *pmoA* 2 and *pmoA* 3). Sequences from cluster *pmoA* 2 did not have any closely related sequences (Fig. 4c).

### Community structures and distribution analyses

Although the seawater and the Rainbow sediment CM-DGGE band patterns were quite similar (Fig. 2a), all the sediment clone library community structures were indistinguishable from the combined communities and significantly different ( $P < 0.001$ ) from the seawater (Fig. 2b). Insignificant  $F_{ST}$  and  $P$  tests ( $P < 0.001$ ), based on an analysis at a 97% sequence similarity level, suggested that community structures from all the Rainbow hydrothermal fluids and Ashadze chimney 1 clone libraries were similar and indistinguishable from the combined communities (Fig. 2b). However, although the archaeal community structures from all the Rainbow hydrothermal fluids were also from similar lineage distributions, all the Ashadze chimney and fluid samples had significantly different population structures ( $P < 0.001$ ; Fig. 2a and 2b). The archaeal diversity of all the other clone libraries was also significantly different from the seawater clone library ( $P < 0.001$ ), showing that the hydrothermal vent archaeal communities are probably adapted to their environment. According to pH measurements, the archaeal diversity in the hydrothermal fluids was always the most reduced in the less diluted fluids (Fig. 2b). Moreover, a correlation was also observed between *Methanococcales* ( $P < 0.001$ ) and *Thermococcales* ( $P < 0.05$ ) lineages and the Rainbow fluids. Correlations were also shown between MG-I lineage and the hydrothermal sediments ( $P < 0.01$ ), and between the unaffiliated *Methanosarcinales* cluster and the Lost City fluids ( $P < 0.0001$ ).

## Discussion

### High diversity of putative chemolithoautotrophs

Overall, the analysis of the phylogenetic data showed a specific distribution of different putative metabolic processes over the different MAR ultramafic-hosted hydrothermal environments that were mainly dominated by putative chemolithoautotrophs.

*Putative ammonia-oxidizing Crenarchaeota*. Marine Group I (MG-I) was the most ubiquitous lineage found in the MAR ultramafic-hosted hydrothermal environments, as sequences related to the MG-I *Archaea* were detected in the majority of clone libraries (93%).

Interestingly, the archaeal community structure of the seawater clone library was dominated by sequences related to MG-I (41%) and Marine Group II (48%), but was significantly different from all the other clone libraries ( $P < 0.001$ ). Congruently, Takai and colleagues showed that the highest proportion of MG-I members in a hydrothermal environment from the Central Indian Ridge, was found in the seawater adjacent to the hydrothermal emissions (Takai *et al.*, 2004c). MG-I sequences also dominated the sediment 16S rRNA gene clone libraries ( $\geq 86\%$ ), as commonly found in marine surface sediments (Inagaki *et al.*, 2001; Teske & Sorensen, 2008; Roussel *et al.*, 2009b). The highest diversity indices were also observed in these sediment samples, as a consequence of the very high intra-phylum diversity observed within the MG-I. However, all the MG-I 16S rRNA gene sequences from the sediment clustered in phylogenetic groups different from the seawater MG-I, suggesting that specific MG-I communities could be associated with sedimentary environmental conditions (Roussel *et al.*, 2009b). Moreover, the G + C content of all the MG-I sequences ranged between 48% to 52%, in opposition to the high-GC content of the 16S rRNA gene sequences from hydrothermal fluids or chimneys, thus supporting the hypothesis that these MG-I *Archaea* are probably adapted to the cooler ecological niches of the hydrothermal environments (Kimura *et al.*, 2006; Ehrhardt *et al.*, 2007). Several studies also showed that specific phylogenetic groups of MG-I *Archaea* appear to be endemic to basaltic crust

environments (Ehrhardt *et al.*, 2007; Mason *et al.*, 2007; Mason *et al.*, 2009). Some of these specific subclades of MG-I *Archaea* could therefore be adapted to various environments as they were also detected in aerobic and anaerobic basalt enrichment cultures and sediment slurries (Mason *et al.*, 2007; Webster *et al.*, 2010). Hence, as the MG-I *Archaea* were widespread in our hydrothermal fluid and chimney clone libraries, their presence could be the result of the mixing of ambient seawater with cool niche water in the rocks of the hydrothermal system.

MG-1 *Archaea*, regularly described as aerobic autotrophic ammonia oxidizers (Francis *et al.*, 2005; Konneke *et al.*, 2005; Hallam *et al.*, 2006), are commonly found in seawater and marine sediments, forming several phylogenetic clusters with several cultured relatives (e.g. Preston *et al.*, 1996; Konneke *et al.*, 2005). Moreover, based on the analysis of the first sequenced genome of a cultured relative (*Cenarchaeum symbiosum*), the MG-1 were proposed as a novel archaeal phylum named *Thaumarchaeota* (Brochier-Armanet *et al.*, 2008). Interestingly, moderate thermophilic ammonia-oxidizing crenarchaeotes were recently isolated from hot springs (de la Torre *et al.*, 2008; Hatzenpichler *et al.*, 2008) and may also play a major role in the nitrogen cycle in these environments (Zhang *et al.*, 2008; Wang *et al.*, 2009). High ammonium concentration and removal rates were previously measured from a Pacific hydrothermal system (Lam *et al.*, 2008), and a thermophilic origin for anaerobic ammonium oxidation was also suggested (Canfield *et al.*, 2006). Hence, according to their widespread dissemination in hydrothermal systems, as shown in several studies, and due to the high mixing processes occurring in these dynamic systems (e.g. Takai *et al.*, 2004c), MG-I may also play a role in ammonium oxidation in hydrothermal systems as previously suggested for marine basalts (Mason *et al.*, 2007; Mason *et al.*, 2009).

*Putative hydrogen-oxidizing chemolithoautotrophs.* All the ultramafic hydrothermal fluids from Rainbow, Ashadze and Lost city were highly enriched in abiogenic methane and hydrogen as a result of serpentinization reactions between ultramafic rocks and seawater (Holm & Charlou, 2001; Charlou *et al.*, 2002; Allen & Seyfried, 2004). Moreover, McCollom showed

that ultramafic-hosted hydrothermal systems could theoretically provide twice as much chemical energy as comparable basaltic-hosted systems (McCollom, 2007). More than half of the archaeal lineages detected from Rainbow, Ashadze and Lost City were related to known cultured species, two thirds of which were involved in hydrogen or methane cycling processes, supporting the theory that these ecosystems could be mainly fuelled by hydrothermal fluids highly enriched in hydrogen and methane.

Methanogenesis was the most common putative hydrogen-oxidizing metabolism detected among ultramafic hydrothermal fluids and chimney samples. Indeed, among all archaeal lineages in the hydrothermal samples, *Methanococcales* was the most widespread, as it was detected in a majority of the clone libraries (78%) obtained from hydrothermal fluids or chimney samples. Interestingly, all the sequences related to *Methanococcales* from Rainbow and Ashadze grouped with sequences previously detected at Rainbow (Nercessian *et al.*, 2005), suggesting that these could be long-term stabilized population in these chemically slow evolving environments (Charlou *et al.*, 2002). *Methanococcales* Archaea are strictly anaerobic autotrophic methanogens, using hydrogen and carbon dioxide or formate as energy sources (Whitman *et al.*, 2001). Strains affiliated to *Methanocaldococcus infernus* were successfully cultured from hydrothermal chimney samples from Rainbow and Ashadze (Jeanthon C. and L'Haridon S. personal communication, respectively). Moreover, the methanogenic potential of *Methanococcales* at Rainbow and Ashadze was also confirmed by the detection of *mcrA* genes related to *Methanothermococcus thermolithotrophicus* (> 97% similarity) and *Methanocaldococcus infernus* (> 97%). Although hyperthermophilic or thermophilic members of the *Methanococcales* are commonly cultured and detected with molecular tools from marine hydrothermal vent systems (e.g. Kelley *et al.*, 2002; Nercessian *et al.*, 2003; Schrenk *et al.*, 2003; Takai *et al.*, 2004a; Perner *et al.*, 2007; Page *et al.*, 2008), Schrenk and colleagues reported that *Methanococcales* encompassed a low proportion (<5%) of the hydrothermal prokaryotic communities associated with the walls of a sulphide chimney, whereas on different hydrothermal sites Takai and colleagues reported proportions



up to 76.5% (Schrenk *et al.*, 2003; Takai *et al.*, 2004a). These differences could be linked to environmental factors such as the high hydrogen production from these ultramafic systems, which fuel these communities.

Moreover, putative hyperthermophilic methanogens were also represented by *Methanopyrales*. *Methanopyrales* were rarely detected on the MAR by molecular methods (Flores *et al.*, 2011), probably as a consequence of technical biases or of the restricted number of microbial studies of the MAR, though the first isolated member originates from a hydrothermal system north of Iceland (Kurr *et al.*, 1991). Sequences related to *Methanopyrales* were rarely detected elsewhere (Nercessian, 2003; Takai *et al.*, 2004a; Ehrhardt *et al.*, 2007; Page *et al.*, 2008). However, in this study, eighteen sequences related to *Methanopyrus kandleri* (>96% similarity) were detected from Rainbow and Ashadze fluids. Interestingly, Takai and colleagues also reported recently an isolate related to *Methanopyrus kandleri* capable of methanogenesis with H<sub>2</sub>/CO<sub>2</sub> under elevated hydrostatic pressures and at 122°C (Takai *et al.*, 2008). As *mcrA* gene sequences related to *Methanopyrus kandleri* (88% similarity) were also detected at Rainbow and as strains affiliated to *Methanopyrales* were successfully cultured from Rainbow and Ashadze (Jeanthon and L'Haridon personal communication, respectively), the *Methanopyrales* detected were probably capable of methanogenesis, thus supporting the hypothesis that these sites may harbour large methanogenic communities.

*Archaeoglobales*, another putative hydrogen-oxidizing archaeal lineage, was also found to be widespread among hydrothermal samples from Rainbow and Ashadze. 16S rRNA gene sequences closely related (> 96% similarity) to members of genus *Archaeoglobus*, *Geoglobus* and *Ferroglobus*, were retrieved from Rainbow and Ashadze. Interestingly, a new dissimilatory Fe(III)-reducing *Archaeoglobaceae* was also isolated from Ashadze and reported as growing autotrophically on hydrogen (Slobodkina *et al.*, 2009). As several *Archaeoglobales* are also iron-cycling *Archaea* (e.g. Kashefi *et al.*, 2002), the high concentrations of iron (> 3 mM) released by acidic ultramafic-hosted hydrothermal environments could possibly fuel specific members of these *Archaeoglobaceae* communities.

Putative sulphur-cycling and methane-oxidizing communities. Members of the *Archaeoglobales* lineage also belong to the hyperthermophilic sulfate-reducing *Archaea* (Miroshnichenko & Bonch-Osmolovskaya, 2006). Interestingly, putative sulphur-cycling *Archaea* related to *Thermococcales* and *Archaeoglobales* lineages were detected in more than half of the clone libraries (56%) obtained from hydrothermal samples (fluid or chimney) and were always detected together, suggesting they could require similar environmental conditions. However, contrary to all Rainbow hydrothermal fluid samples, *Thermococcales* and *Archaeoglobales* at Ashadze were only detected from the Ashadze chimney 1 samples, suggesting that all hydrothermal vents from Ashadze site did not share optimal conditions for putative sulphur-cycling microorganisms. Interestingly, the first obligate piezophilic hyperthermophilic microorganism, *Pyrococcus* CH1, was also recently isolated from the Ashadze chimney 1 (Zeng *et al.*, 2009). Members of the *Thermococcales* order are mainly characterized as thermophilic to hyperthermophilic anaerobic heterotrophs that ferment peptides and sugars, and their growth can also be stimulated by sulphur reduction (Miroshnichenko & Bonch-Osmolovskaya, 2006; Zeng *et al.*, 2009). However, some members of the *Thermococcales* were also able to grow on acetate-utilising Fe(III) (Summit & Baross, 2001) or capable of lithotrophic growth on carbon monoxide coupled with hydrogen production (Sokolova *et al.*, 2004), thus matching the environmental conditions of ultramafic-hosted hydrothermal systems. It has also been suggested that *Thermococcales* and hyper/thermophilic members of the *Methanococcales* order could inhabit sub-seafloor ecosystems (Summit & Baross, 1998; Summit & Baross, 2001; Kelley *et al.*, 2002; Takai *et al.*, 2004a), and could be part of a hydrogen-driven subsurface lithoautotrophic microbial ecosystem (Nealson *et al.*, 2005).

Within methane cycling communities associated with Rainbow, putative methanotrophic ANME-2 sequences were detected, suggesting occurrence of anaerobic methane oxidation communities associated with anoxic habitats below 90°C (Kallmeyer & Boetius, 2004). Interestingly, *dsrA* gene sequences detected at Ashadze clustered with sequences

previously detected in methane-rich hydrothermal systems and related to the *Desulfobulbaceae* family (Teske *et al.*, 2002; Nercessian *et al.*, 2005), indicating that these putative sulfate-reducing bacteria could be linked to these specific environmental conditions. Besides, some members of *Desulfobulbaceae* can live syntrophically with ANME-3 members (Niemann *et al.*, 2006). However, although 16S rRNA gene sequences related to ANME-2 *Archaea* were detected, no ANME-3 *Archaea* were found. Nevertheless, the most widespread functional gene (*pmoA*) detected in ultramafic-hosted hydrothermal environments remained related to methanotrophic communities as a probable consequence of the high methane concentration prevailing in these ultramafic-hosted hydrothermal systems. Methanotrophic bacteria were also previously detected in *Bathymodiolus* species and among the gill chamber of *Rimicaris exoculata* at the Rainbow hydrothermal field (Duperron *et al.*, 2006; Zbinden *et al.*, 2008), suggesting that these symbionts could also be present in seawater. However, no known sequences related to symbionts were detected in the seawater, as a possible consequence of low cell concentrations or of a technical bias. Moreover, the phylogenetic distribution of the *pmoA* gene was related to the habitat, suggesting that different methanotrophic communities were specifically adapted to different ecological niches (e.g. sediments and fluid/seawater mixing zones).

### **Specific distribution and ecological niches**

The different environmental conditions (temperature, pH, hydrostatic pressure, metabolic substrates) at the different MAR ultramafic-hosted hydrothermal sites generate diverse microbial ecological niches (hydrothermal fluid, chimney, sediment, and seawater) that seem to strongly select for specific communities.

*Site specific phylotypes.* Although molecular techniques (PCR and cloning) used to build clone libraries are known to be inherently biased (Suzuki & Giovannoni, 1996; von Wintzingerode *et al.*, 1997; Polz & Cavanaugh, 1998; Nocker *et al.*, 2007), we assumed that the biases were equal for all samples as they were analyzed under the same strict conditions (storage, DNA extraction, PCR amplification, cloning, sequencing) (von Wintzingerode *et al.*,

1997). However, comparisons of population structures from other studies using different experimental conditions remain unreliable. For example, archaeal diversity from Rainbow chimneys as described by Flores and colleagues (2011) was much higher than that described by Voordeckers and colleagues (2008), suggesting either spatial and temporal heterogeneity or a technical bias. Interestingly, archaeal diversity observed from the Rainbow fluids in the present study was similar to that from the chimneys analysed using pyrosequencing reported by Flores and colleagues (2011). The present study also shows that some communities seemed to be site-specific and specifically adapted to different ecological niches (e.g. sediments and fluid/seawater mixing zones).

Sequences related to *Nanoarchaeota*, for example, were only detected in the Rainbow hydrothermal system, showing that the nanoarchaeal habitat extends to at least one of the deep hot marine hydrothermal systems of the MAR. The recently discovered novel *Nanoarchaeota* phylum has shown a wide distribution in high temperature ecosystems (Hohn *et al.*, 2002; Huber *et al.*, 2002), and may represent pioneering communities in deep-sea hydrothermal vents (McCliment *et al.*, 2006). *Nanoarchaeota* could also represent a fast-evolving euryarchaeal lineage related to *Thermococcales* (Brochier *et al.*, 2005). Moreover, the nano-sized *Nanoarchaeota* were previously described to have a symbiotic relationship with *Ignicoccus hospitalis*, a member of the *Desulfurococcales* order isolated from the Kolbeinsey Ridge, north of Iceland (Paper *et al.*, 2007). Interestingly, 16S rRNA gene sequences with 94% similarity to the hyperthermophilic chemolithoautotrophic sulphur and hydrogen-utilizing *Ignicoccus hospitalis*, were also retrieved exclusively from the same Rainbow hydrothermal fluids, suggesting that a similar symbiotic relationship could also occur between the *Nanoarchaeota* and specific *Desulfurococcales* from Rainbow.

Differences between the composition of archaeal communities associated with the two hydrothermal chimneys from Ashadze could be probably linked to environmental factors as the Ashadze chimney 3 has a higher copper concentration than chimney 1 (Charlou JL., Donval JP. and Konn C., unpublished). Moreover, 16S rRNA gene sequences related to

*Halobacteriales* were only detected from Ashadze, which is to date the deepest known hydrothermal site. The highest similarity with a cultured relative was *Natronomonas pharaonis* (98%), an extremely halo-alkaliphilic archaeon. The occurrence of halotolerant prokaryotes in hydrothermal environments, growing at higher NaCl concentrations than most marine microorganisms, was previously reported (Takai *et al.*, 2001). Due to phase separation it is admitted that venting of a condensed vapor phase with low salinity will generate a high salinity phase at depth. This phase may be venting later or be trapped in the subsurface environments. In addition some authors have suggested a double diffusive hydrothermal system where brines are trapped in the deepest part of the system and exchange only heat with the upper convective system (Bischoff & Rosenbauer, 1989; Fouquet *et al.*, 1993). If partially cooled, this deep high salinity reservoir may constitute an extensive location for halotolerant prokaryotes. Hence, it was also suggested that these communities could be associated with a sub-vent ecosystem, as well as with hydrothermal chimneys (Kaye & Baross, 2000; Takai *et al.*, 2001).

As previously described, the off-axis Lost City hydrothermal system is remarkable by its geological, geochemical and biological settings (Kelley *et al.*, 2005). The archaeal diversity associated with hot and very alkaline Lost City hydrothermal fluid was limited to unaffiliated *Methanosarcinales* and to MG-I sequences. The detected unaffiliated *Methanosarcinales* sequences matched the Lost City *Methanosarcinales* cluster (99% similarity) described by Schrenk and colleagues (Schrenk *et al.*, 2004), suggesting that these *Archaea* were involved in methane cycling processes (Schrenk *et al.*, 2004; Boetius, 2005). However, no *mcrA* gene sequences were detected at Lost City, as a likely consequence of low cell densities in the Lost City fluids (Brazelton *et al.*, 2006). Members of the Lost City uncultured *Methanosarcinales* cluster are probably endemic communities associated to cooler (<95°C) and very alkaline habitats as they were not detected from any other hydrothermal sites. The occurrence of molecular genetic evidences in hot and very alkaline fluids also suggests that the Lost City *Methanosarcinales* have physiological potentials beyond the capacities of any known cultured isolates (Mesbah & Wiegel, 2008).

*Specific ecological niches.* To summarize, besides some specific *Archaea* that seemed endemic to some hydrothermal sites, the distribution of archaeal phylotypes and putative metabolic processes was linked to different microbial niches (seawater, sediments, macrofaunal communities, hydrothermal chimneys and fluids, Fig. 5). The cold and oxygenated seawater (< 10°C) overlaying the hydrothermal systems probably represented one of the largest microbial niches, and was characterized only by marine group lineages, some of which could be aerobic ammonia oxidizers. These psychrophilic seawater communities surrounding hydrothermal vents are most likely to benefit from high ammonium inputs from the chemolithotrophic primary producer associated with the hydrothermal structures. Another large ecological niche is probably the cold (< 10°C) and porous sediments surrounding the hydrothermal systems, which may represent a stable environment and suitable substrate for selection of specific seawater phylotypes (MG-I) and for colonization by specific psychrophilic unaffiliated *Euryarchaeota* and methylotrophic bacteria. The specific sedimentary microbial communities could be fuelled by the products from organic matter degradation, but also by methane seepage from these ultramafic systems. In contrast, the warm (~15°C) and relatively unstable mixing zones colonized by macrofaunal communities were probably the most metabolically active microbial niche benefiting from oxidized seawater compounds and from reduced compounds from the hydrothermal system. Mixing zones between the adjacent ecological niches also occur as a result of steep physico-chemical gradients characterizing these dynamic hydrothermal environments, therefore resulting in exchanging microbial communities. Mesophilic to thermophilic methane-oxidizing bacteria could dominate the moderate oxic habitats in the mixing environment as revealed by the *pmoA* gene analyses. The detection of ANME-2 members suggests that moderate thermophilic (<90°C) anaerobic methanotrophs could occur in probably restricted anoxic habitats, as a consequence of the very steep oxygen and temperature gradients. Methanotrophic archaeal communities fuelled by hydrogen and carbon dioxide could probably dominate the more chemically reduced zones of this niche, which is closer to the hydrothermal chimney. The thermophilic communities composed of *Methanococcales*,

*Methanopyrales*, *Thermococcales*, *Archaeoglobales*, and *Desulfurococcales* were in all likelihood harboured by the hydrothermal chimneys and could mainly be composed of hydrogen-oxidizing members. Although hydrothermal fluids from ultramafic systems such as Rainbow do not have significant levels of hydrocarbons from biogenic origin, methanogenesis could still be the dominant archaeal metabolic process, as the high abiogenic methane concentration may mask the biogenic methane. Moreover, the hyper/thermophilic methanogen *Methanococcales* order and the *Thermococcales* order could be typical members of the hot anaerobic microbial ecosystem that could extend below the Rainbow hydrothermal system seafloor.

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## 984 Captions

985 **Fig. 1.** (a) Location map of Atlantic ultramafic-hosted hydrothermal sites mentioned in this

986 study. (b) Photograph of the sediment sampling at Lost City hydrothermal field. (c)

987 Photograph of the fluid sampling using titanium syringe at Lost City hydrothermal field. (d).

988 Photograph of the sediment sampling using push-core devices at Rainbow hydrothermal

989 field. (e). Photograph of the fluid sampling using titanium syringe at Rainbow hydrothermal

990 field. (f). Photograph of temperature measurements at Ashadze 1 hydrothermal field.

991 **Fig. 2.** (a) Co-migration denaturant gradient gel electrophoresis (CM-DGGE) analysis of

992 archaeal 16S rRNA genes from seawater (blue) compared to Rainbow, Ashadze and Lost

993 City hydrothermal environments (red). The white arrows indicate the position of faint DGGE

994 bands. PCR products were amplified with the Saf-PARCH 519r\*Cy5 (blue) or Saf-PARCH

995 519r\*Cy3 (red) primer set and electrophoresis was performed using a gradient of 30–70%

996 denaturant. (b) Distribution of the archaeal phylogenetic communities based on 16S rRNA

997 gene from three ultramafic-hosted hydrothermal sites. The phylogenetic affiliation of each

998 clone sequence was determined by similarity analysis. For each phylogenetic affiliation, the

999 average G + C content of the detected 16S rRNA gene sequences is shown in brackets. The

1000 relative abundance of each phylotype was calculated and represented in a column diagram.

1001 Cx indicates coverage percentage for each clone library. OTU indicates the number of

1002 operational taxonomic units (95%) for each clone library. SW indicates the Shannon-Wiener

1003 index of diversity. *dsr*, *pmo* and *mcr*, respectively indicate positive amplification of the

1004 functional genes. ND: not determined. The asterisks indicate groups of clone libraries with

1005 insignificant ( $P < 0.001$ ) differences between all the diversity indices ( $F_{ST}$  and the exact test

method). ANME-2: *anaerobic methane* oxidizers, DHVE2: Deep-sea Hydrothermal Vent *Euryarchaeota*, MBG-D: Marine Benthic Group D, MBG-A: Marine Benthic Group A, MG-1 (II, III, IV): Marine Group 1 (II, III, IV), MBG-E: Marine Benthic Group E, UHE-1: Unaffiliated Hydrothermal *Euryarchaeota*.

**Fig. 3.** (a) Phylogenetic tree representing the *Euryarchaeota* 16S rRNA gene sequences. Each phylotype is represented by one sequence with  $\geq 97\%$  similarity grouping. The tree was constructed using the Neighbor-Joining method with Jukes and Cantor correction. Bootstrap values  $< 50\%$  are not shown. Circles symbolize Ashadze clone libraries. Triangles symbolize Rainbow clone libraries. Squares symbolize Lost City clone libraries. Underlined sequences: seawater clone library. ANME: *anaerobic methane* oxidizers, DHVE: Deep-sea Hydrothermal Vent *Euryarchaeota*, MBG-D: Marine Benthic Group D, MBG-E: Marine Benthic Group E, SAGMEG: South African Gold Mine *Euryarchaeotic* Group, UHE-1: Unaffiliated Hydrothermal *Euryarchaeota*. (b) Phylogenetic tree representing the *Crenarchaeota* 16S rRNA gene sequences. Each phylotype is represented by one sequence with  $\geq 97\%$  similarity grouping. The tree was constructed using the Neighbor-Joining method with Jukes and Cantor correction. Bootstrap values  $< 50\%$  are not shown. Circles symbolize Ashadze clone libraries. Triangles symbolize Rainbow clone libraries. Squares symbolize Lost City clone libraries. Underlined sequences: seawater clone library. MCG: Miscellaneous *Crenarchaeotal* Group, MBG-B: Marine Benthic Group B, MBG-A: Marine Benthic Group A.

**Fig. 4.** Phylogenetic trees based on translated partial amino acid sequences of functional genes (*mcrA*, *dsrA*, *pmoA*). The trees were constructed using the Neighbor-Joining method using PAM distance (Dayhoff *et al.*, 1978). Bootstrap values  $< 50\%$  are not shown. Circles symbolize Ashadze clone libraries. Triangles symbolize Rainbow clone libraries. Squares symbolize Lost City clone libraries. (a) *mcrA* gene. (b) *dsrA* gene. (c) *pmoA* gene.

**Fig. 5.** Hypothetical model (not to scale) of microbial ecological niches in acidic Atlantic ultramafic-hosted hydrothermal systems (Rainbow, Ashadze). Each ecological niche was described by its average temperature, potential electron donors and acceptors metabolized



1033 by the microbial communities described in this study, and the distribution of these microbial  
1034 communities. OM: Organic matter. \*Lineage only detected from Ashadze.

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## 1037 **Supplementary material**

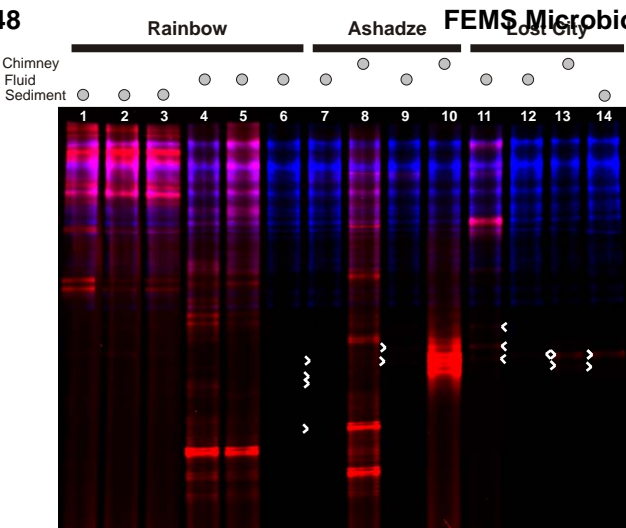
1038 **Fig. S1.** Rarefaction curves for the 16S rRNA gene clone libraries from the Fairway and New  
1039 Caledonia Basin sites (Schloss and Handelsman, 2005). The sequence identity levels are  
1040 represented in brackets.

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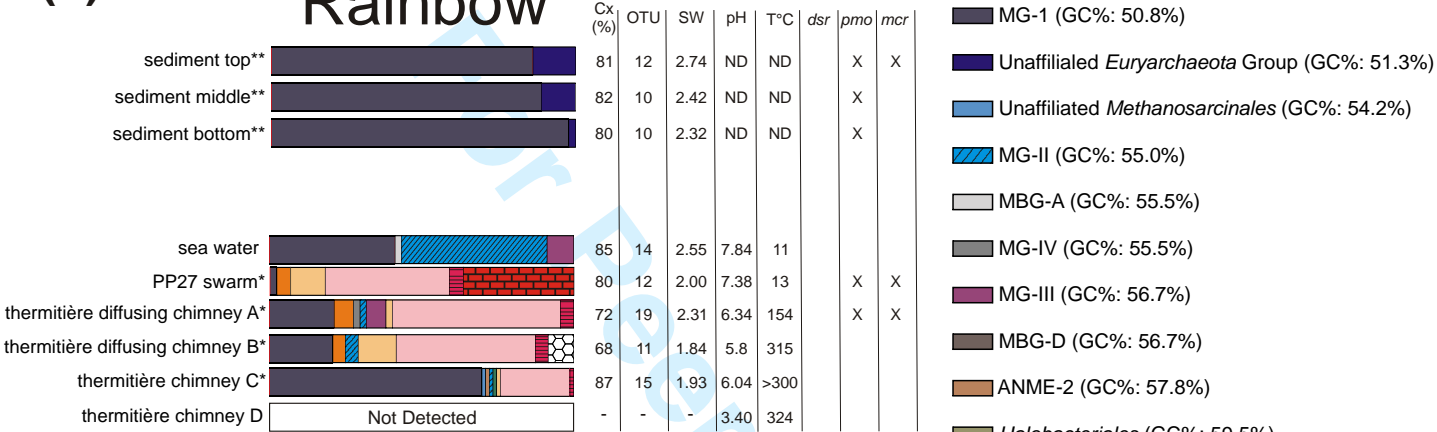




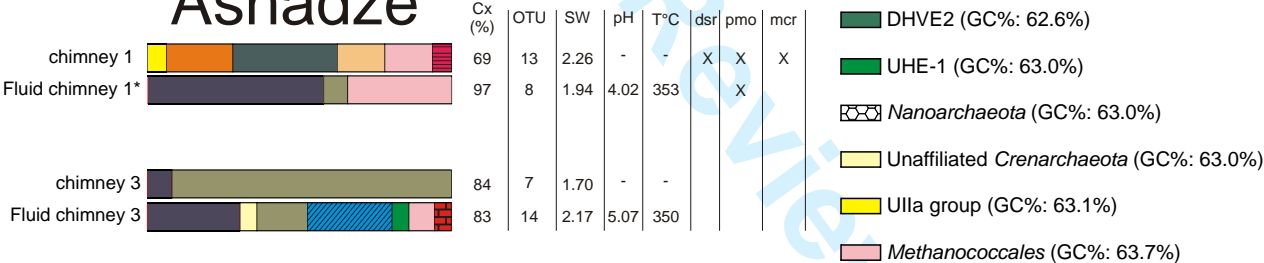
- 1 Rainbow, sediment top + Seawater
- 2 Rainbow, sediment middle + Seawater
- 3 Rainbow, sediment bottom + Seawater
- 4 Rainbow, PP27 swarm + Seawater
- 5 Rainbow, thermitière diffusing chimney A + Seawater
- 6 Rainbow, thermitière diffusing chimney B + Seawater
- 7 Ashadze, Fluid chimney 1 + Seawater
- 8 Ashadze, chimney 1 + Seawater
- 9 Ashadze, Fluid chimney 3 + Seawater
- 10 Ashadze, chimney 3 + Seawater
- 11 Lost City, Fluid (pH 10.66) + Seawater
- 12 Lost City, Fluid (pH 11.75) + Seawater
- 13 Lost City, chimney + Seawater
- 14 Lost City, sediment + Seawater

(b)

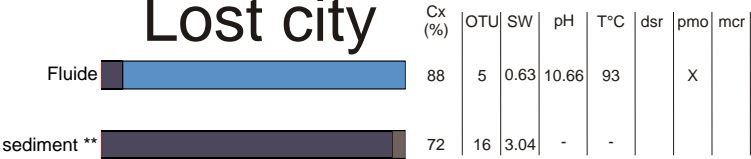
**Rainbow**

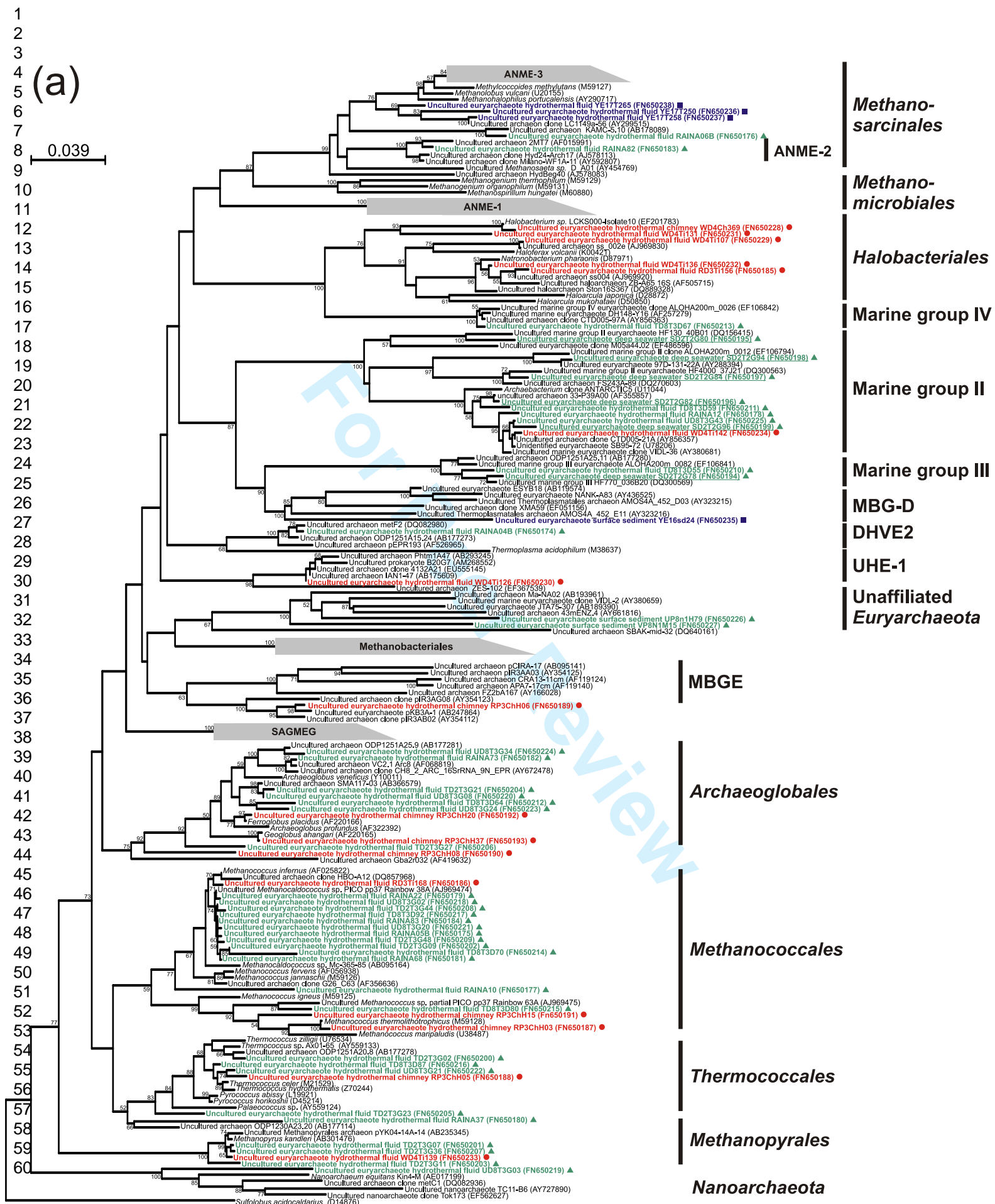


**Ashadze**

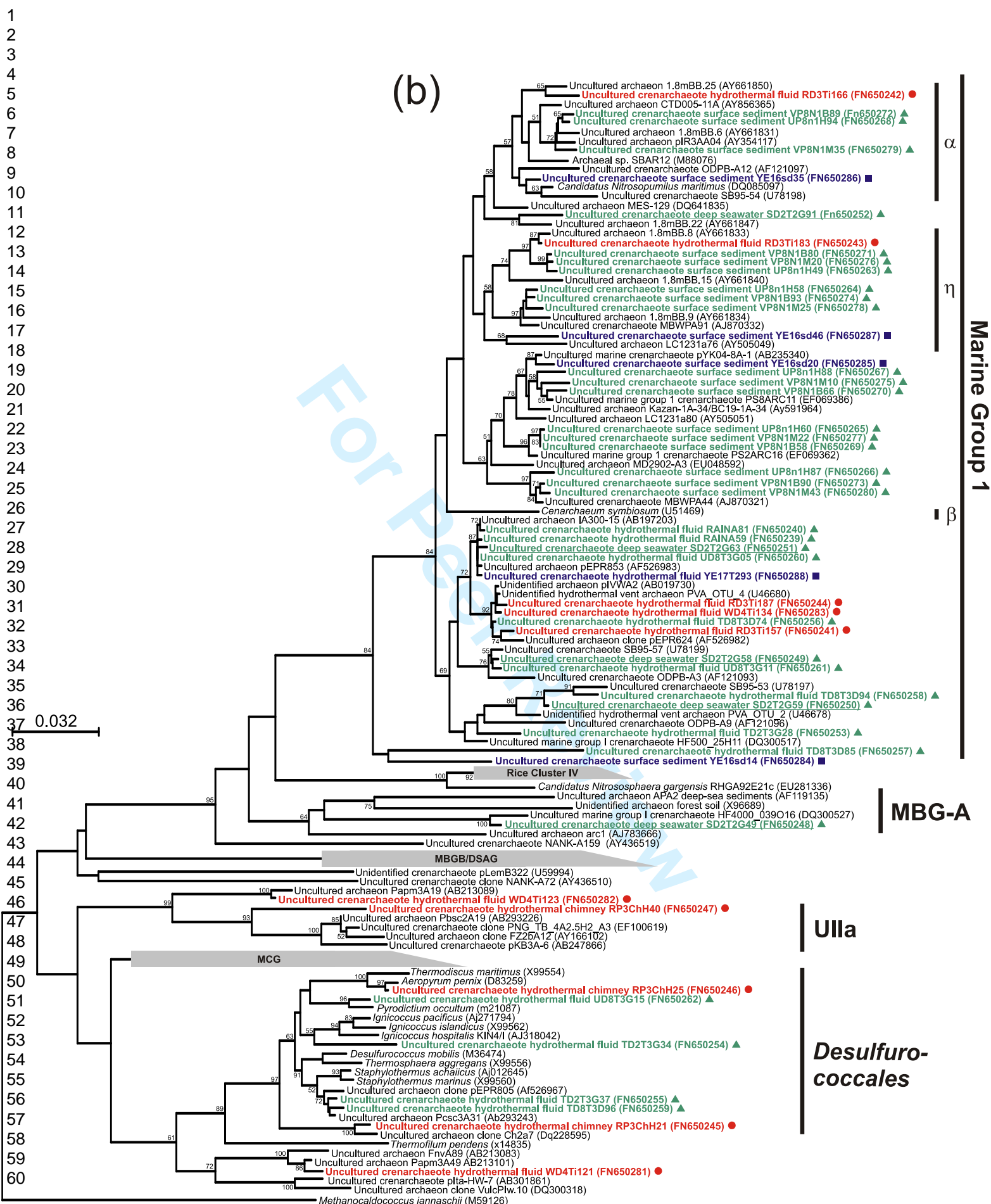


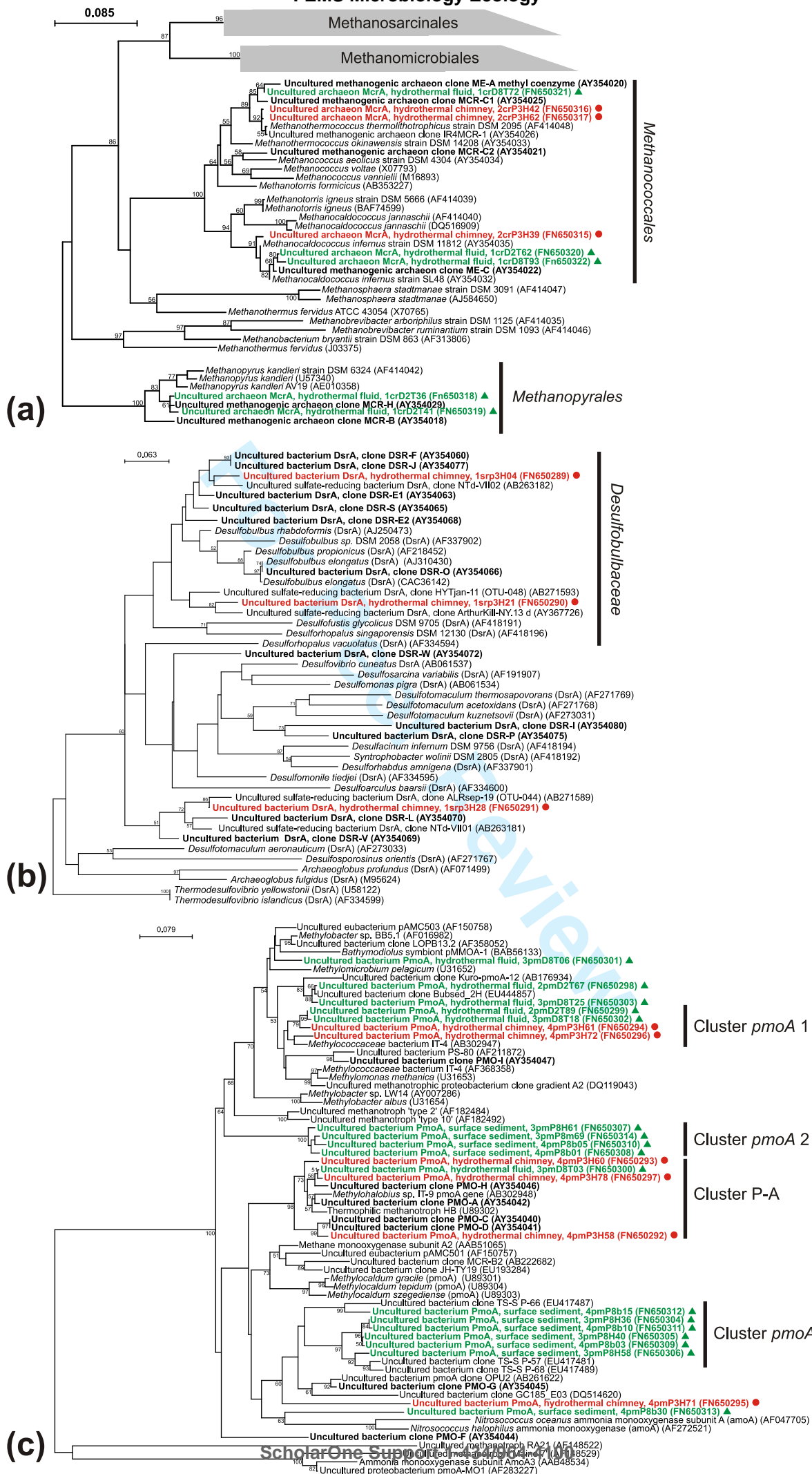
**Lost city**



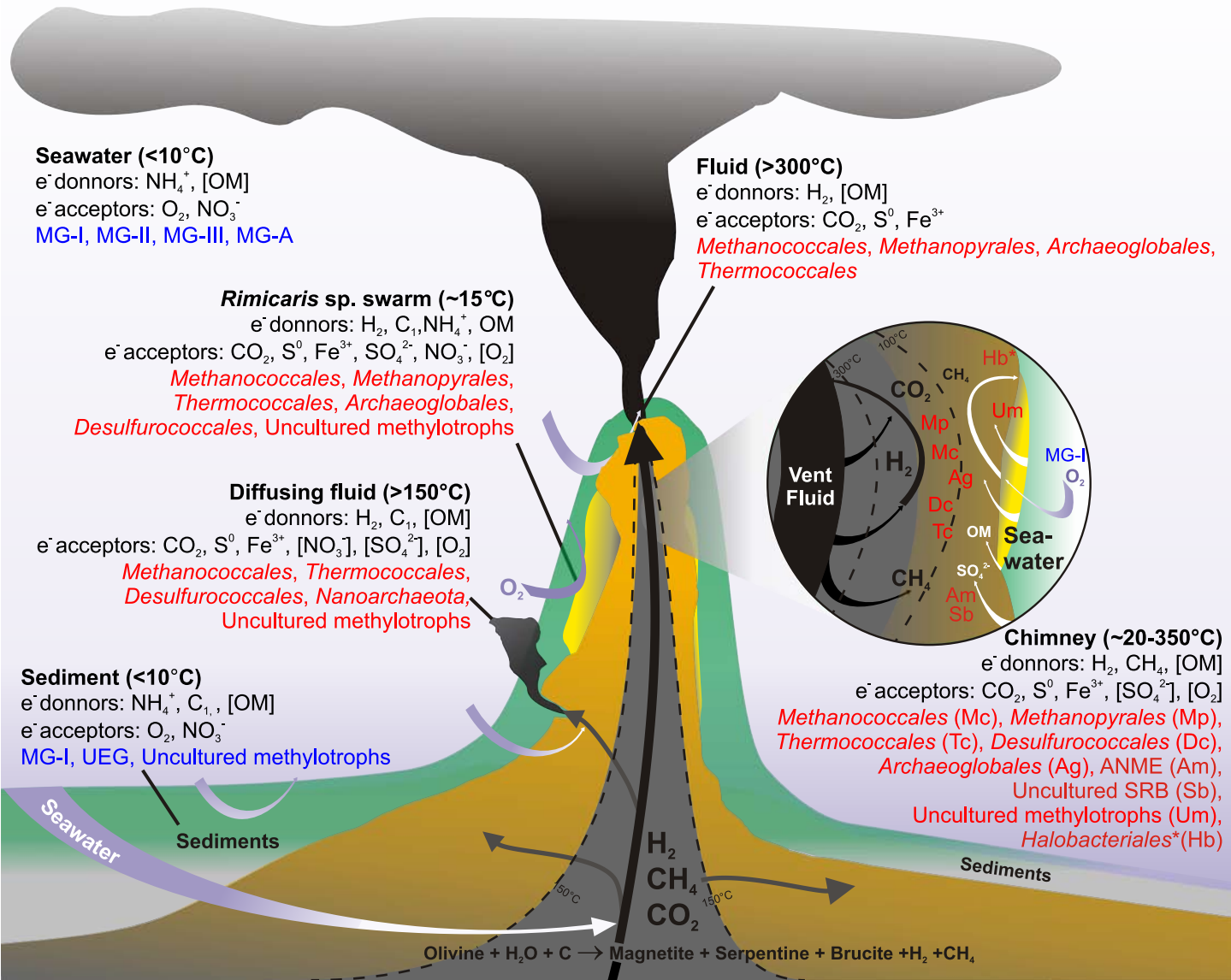








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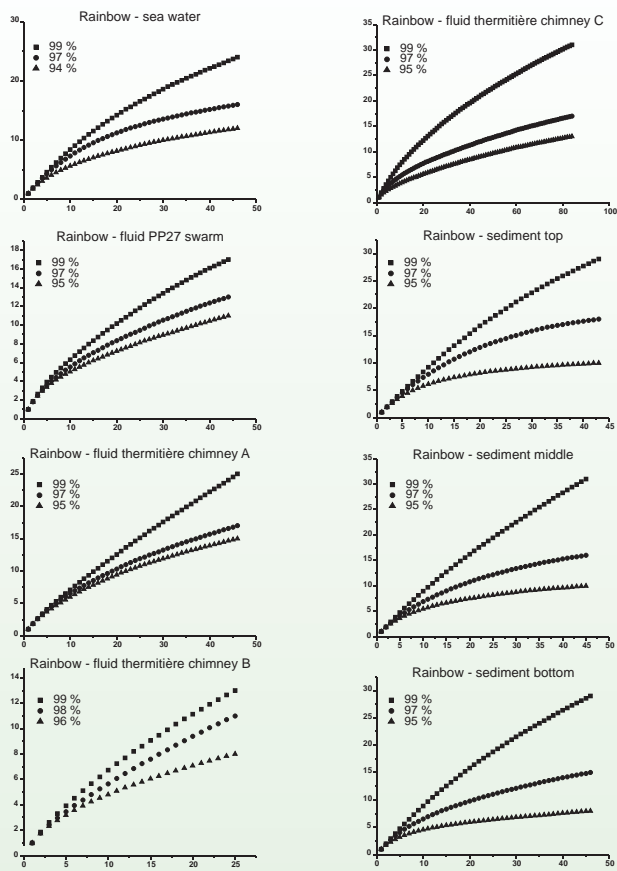


- Macrofaunal communities
- Anaerobic Hydrogen-oxidizing communities
- Aerobic methane-oxidizing communities

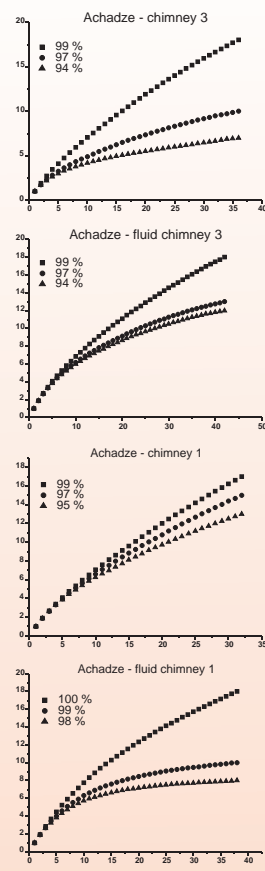
Psychrophilic communities  
Mesophilic to hyperthermophilic communities  
[Variable concentration]

## Supplementary material

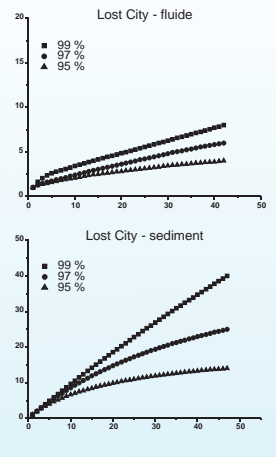
### Rainbow



### Ashadze



### Lost City



**Fig. S1.** Rarefaction curves for the 16S rRNA gene clone libraries from the Fairway and New Caledonia Basin sites (Schloss and Handelsman, 2005). The sequence identity levels are represented in brackets.